Carbohydrates do not accelerate force recovery after glycogen-depleting followed by high-intensity exercise in humans

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Prolonged low-frequency force depression (PLFFD) induced by fatiguing exercise is characterized by a persistent depression in submaximal contractile force during the recovery period. Muscle glycogen depletion is known to limit physical performance during prolonged low- and moderate-intensity exercise, and accelerating glycogen resynthesis with post-exercise carbohydrate intake can facilitate recovery and improve repeated bout exercise performance. Short-term, high-intensity exercise, however, can cause PLFFD without any marked decrease in glycogen. Here, we studied whether recovery from PLFFD was accelerated by carbohydrate ingestion after 60 minutes of moderate-intensity glycogen-depleting cycling exercise followed by six 30-seconds all-out cycling sprints. We used a randomized crossover study design where nine recreationally active males drank a beverage containing either carbohydrate or placebo after exercise. Blood glucose and muscle glycogen concentrations were determined at baseline, immediately post-exercise, and during the 3-hours recovery period. Transcutaneous electrical stimulation of the quadriceps muscle was performed to determine the extent of PLFFD by eliciting low-frequency (20 Hz) and high-frequency (100 Hz) stimulations. Muscle glycogen was severely depleted after exercise, with a significantly higher rate of muscle glycogen resynthesis during the 3-hours recovery period in the carbohydrate than in the placebo trials (13.7 and 5.4 mmol glucosyl units/kg wet weight/h, respectively). Torque at 20 Hz was significantly more depressed than 100 Hz torque during the recovery period in both conditions, and the extent of PLFFD (20/100 Hz ratio) was not different between the two trials. In conclusion, carbohydrate supplementation enhances glycogen resynthesis after glycogen-depleting exercise but does not improve force recovery when the exercise also involves all-out cycling sprints.

KEYWORDS
central fatigue, force recovery, high-intensity interval training, maximal voluntary contraction, muscle torque, peripheral fatigue, prolonged low-frequency force depression, skeletal muscle
1 | INTRODUCTION

Prolonged low-frequency force depression (PLFFD) describes a phenomenon during the post-exercise recovery period in which submaximal (low-frequency) contractile force is depressed to a greater extent than maximal force. PLFFD can persist for hours if not days following fatigue-inducing exercise. Given that exercise normally involves submaximal contractions, PLFFD has particular functional relevance to sports performance and can likely explain the feeling of muscle weakness that can persist hours after fatiguing exercise. Mechanisms within the skeletal muscle fibers emerge as the primary causes of PLFFD because in addition to PLFFD being observed in human studies, PLFFD has also been shown in fatigued isolated rodent muscle and single human muscle fibers. Accordingly, impaired sarcoplasmic reticulum (SR) Ca2+ release and/or decreased myofibrillar Ca2+ sensitivity have been implicated as the primary causes of PLFFD. Recent studies show that exercise-induced increases in reactive oxygen and nitrogen species (RONS) are involved in the development of PLFFD. Antioxidant treatments do not prevent PLFFD, but tend to shift the cause from impaired SR Ca2+ release to decreased myofibrillar Ca2+ sensitivity.

It is well known that glycogen, which is a major fuel for muscle contraction, is readily depleted following prolonged endurance exercise. Similarly, enhanced muscle glycogen resynthesis with carbohydrate supplementation is known to accelerate recovery after exhaustive exercise. Depletion of glycogen mainly from the intra-myofibrillar pools close to the SR has been associated with impaired SR Ca2+ release in elite cross-country skiers after exhaustive endurance-type exercise, as well as in mechanically skinned rat fibers and intact mouse fibers following fatiguing contractions. In addition, enzymatic removal of glycogen in unfatigued mechanically skinned rat fibers resulted in impaired SR Ca2+ release. Likewise, glycogen resynthesis affects the recovery of SR Ca2+ release and the reversal of PLFFD in mouse single muscle fibers: fibers superfused with glucose showed a greater recovery of low-frequency (30 Hz) force than fibers recovering in the absence of glucose, whereas high-frequency (120 Hz) force recovered rapidly both with or without glucose. Thus, the results of previous studies indicate that development of and recovery from PLFFD can depend on muscle glycogen depletion and restitution, respectively.

Prolonged low-frequency force depression has also been observed after short-term high-intensity exercise. Such short-lasting types of exercise will not result in any major reduction in muscle glycogen. For instance, four 30-seconds all-out cycling sprints resulted in only ~20% reduction in muscle glycogen content. Thus, the PLFFD observed after short-term high-intensity exercise must depend on other mechanisms than glycogen depletion and one likely candidate is increased RONS production. Mitochondrial RONS production increases during exposure to low O2 levels. The maximal aerobic capacity is substantially exceeded during short-term sprint exercise, hence promoting a hypoxic environment. Accordingly, measurements of RONS with fluorescent indicators show relatively small increases during classical moderate-intensity fatiguing stimulation, whereas an increase of ~200% was observed in isolated mouse muscle fibers exposed the six 30 seconds periods of high-intensity contractions.

The purpose of the current study was to determine whether the recovery of PLFFD induced by prolonged moderate-intensity glycogen-depleting followed by high-intensity sprint cycling exercise is affected by carbohydrate supplementation and consequently glycogen resynthesis. We focused on the acute 3-hours recovery period, which is within the temporal window where the rate of muscle glycogen resynthesis is the highest, and used a randomized crossover study design where male adults received carbohydrate (CARB) or placebo (PLA) supplementation after the cycling exercise. We hypothesized that glycogen resynthesis with CARB supplementation would improve recovery of PLFFD following an exercise task involving prolonged moderate-intensity exercise followed by sprint cycling.

2 | MATERIALS AND METHODS

2.1 | Participants

A randomized crossover study design was used in this study, in which 11 healthy male adults were initially recruited. Inclusion criteria: 18-40 years of age, reportedly healthy with normal blood pressure and no medication, and body mass index (BMI) < 30 kg m⁻². All participants were physically active and performed recreational activities up to 5 hours per week. Exclusion criteria: regular training for more than 5 hours per week, or any disease, injury or another condition that would impose difficulties to conduct the tests. Due to the withdrawal of one subject and the exclusion of another with BMI > 30 kg m⁻², nine participants (30.0 ± 6.0 years) were finally included in the study. The height, body mass, percentage body fat (measured by bio-electrical impedance; Tanita TBF-300 UK Ltd.), BMI, and maximal oxygen uptake (VO2max, as described below) were 185.8 ± 4.9 cm, 80.7 ± 10.9 kg, 14.0 ± 5.1%, 23.4 ± 3.2 kg/m², and 48.5 ± 7.2 mL/kg/min, respectively. All experiments were performed at the Lithuanian Sports University (Kaunas, Lithuania). The study protocol was approved by the Kaunas Regional Research Ethics Committee (no. BE-2-17) and was in agreement with the latest revision of the Declaration of
Helsinki. The participants were informed of the experimental procedures and gave their written informed consent prior to participation.

### 2.2 Experimental design

The experiments consisted of three visits to the laboratory. The temperature in the laboratory was controlled and maintained at 21°C, and a fan was used to keep the subjects comfortable during exercise. The participants did not perform any strenuous exercise during the last 3 days prior to each experimental session. During the first visit, a maximal incremental cycling test was performed to determine VO₂max, which was used to set the submaximal intensity during the subsequent continuous cycling exercise sessions (ie, in visits 2-3). After this cycling test, the participants were also familiarized with the neuromuscular testing and sprint cycling exercises. The first visit was performed approximately a week before the second visit. In visits 2-3 (separated by 4 weeks), the participants performed the two experimental trials (Figure 1). The subjects arrived at the laboratory in the morning after 10-12 hours of overnight fasting, and a first biopsy was collected from the vastus lateralis muscle. The baseline neuromuscular testing was preceded by a warm-up protocol, which consisted of 8-10 minutes of cycling at 1 W/kg body mass, light active dynamic stretching of the quadriceps, and two to three light and short isometric knee extensions in the dynamometry chair. After warm-up, the neuromuscular testing of the knee extensors was performed. Then, the participants completed a glycogen-depleting exercise session (see Section 2.4 below for details). This protocol was adapted from a previous glycogen-depleting exercise protocol where subjects performed a continuous cycling exercise followed by several cycling sprints.²⁴,²⁷ A second biopsy was collected 2-3 minutes after the completion of the last sprint exercise. In a randomized order, the subjects drank a beverage containing either carbohydrate (CARB) or a placebo (PLA) during the post-exercise recovery period (see Section 2.5 below for details). A third muscle biopsy was then collected 180 minutes after the last sprint exercise (ie, 30 minutes after the last beverage was consumed). Capillary blood samples from the fingertip were collected at baseline, immediately after the 60-minutes continuous cycling exercise, immediately after the last cycling sprint, and 180 minutes after the last carbohydrate or placebo ingestion.

**FIGURE 1** Schematic of the experimental session (visits 2-3: two randomized trials). During the pre-exercise period, a warm-up at low intensity (light gray bar) was followed by neuromuscular testing (NM testing), consisting of isometric torques of the knee extensors. During the exercise session, the participants performed a 60-min continuous cycling exercise (darker gray bar) followed by six 30-s all-out cycling sprints (black bars). Neuromuscular (NM) testing was performed at 15, 30, 60, 90, 120, 150, and 180 min after the last exercise session. During the recovery period (from 15-150 min post-exercise), the subjects were given a carbohydrate (CARB) or a placebo (PLA) beverage (2 randomized trials).
sprint and every 30 minutes during the 180-minutes recovery period. The blood concentrations of glucose and lactate were directly measured with portable devices (Glucocard X-mini Plus, and lactate Pro, Arkay Inc.). Neuromuscular testing was performed before exercise and at 15, 30, 60, 90, 120, 150, and 180 minutes of recovery.

2.3 Maximal incremental cycling test

VO\textsubscript{2max} was determined during a maximal incremental cycling test performed on an electromechanically braked cycling ergometer (Ergoselect 200P, Ergoline, Medical Measurement Systems). After a warm-up (8-10 minutes at 1 W/kg body mass), the participants started the test with 3 minutes cycling at 40 W, after which the power increased by 5 W every 10 seconds until exhaustion. The pedaling cadence had to be maintained above 70 rpm. Participants received verbal encouragement throughout the test. Expired gases were collected and analyzed using a breath-by-breath gas analyzer (Oxycon Mobile, Jaeger/VIASYS Healthcare), and heart rate (HR) was measured with a HR monitor (S810, Polar).

Subjects were considered to have reached their VO\textsubscript{2max} when at least three of the following criteria were attained: (a) HR reached at least 90% predicted maximal HR; (b) the respiratory exchange ratio (RER) exceeded 1.1; (c) the VO\textsubscript{2} did not further increase during the subsequent 30 seconds of the test; and (d) the rated perceived exertion (RPE) reached at least 19 on the BORG 6-20 RPE scale. VO\textsubscript{2max} was calculated as the highest 15 seconds value.

2.4 Glycogen-depleting followed by sprint exercise session

The 60-minutes continuous cycling exercise was performed on an electromechanically braked cycling ergometer (Ergoselect 200P, Ergoline, Medical Measurement Systems). The intensity was defined as the power eliciting 60% VO\textsubscript{2max} and was calculated from the power-VO\textsubscript{2} relationship obtained during the maximal incremental cycling test performed at the first visit (see above). The pedaling cadence was set at 70 rpm, participants were allowed to take a 2-3 minutes break after 30 minutes cycling, and they could drink water during the test (up to 0.5 L). A pause was provided to increase exercise tolerability since subjects were not highly trained and continuous cycling for 60 minutes for some of them was not an easy task. Water intake was standardized across test days. HR and the rate of perceived exertion (RPE, 6-20 scale) were determined every 10 minutes to evaluate the exercise intensity.

The subsequent sprint exercise was performed on a mechanically braked cycling ergometer (Monark 824E; Monark) equipped with a basket weight loading system. Six all-out cycling sprints of 30 seconds interspaced with 4 minutes of recovery were completed. After a maximal acceleration without resistance to reach the maximal pedaling cadence, a break weight corresponding to 7.5% of the subject’s body weight was applied to initiate the all-out cycling bout.\textsuperscript{19} HR and power output were averaged every 5 seconds during the six sprints.

2.5 Carbohydrate supplementation and nutritional control

The carbohydrate-enriched beverage contained glucose and fructose at a 2:1 ratio. A mixture of glucose and fructose was ingested in order to increase carbohydrate absorption, improve tolerance, and minimize gastrointestinal distress at high concentrations of carbohydrate consumption.\textsuperscript{28} The carbohydrate was dissolved in a solution of water supplemented with lemon juice concentrate (2.2 g carbohydrate per 100 mL of concentrate; Solevita; 1:20 dilution). The final beverage contained 100 g/L carbohydrate. A placebo beverage was prepared by dissolving 4 g/L of Suketter (containing sodium cyclamate and sodium saccharin; Cederoth) and 0.6 g/L of sodium saccharin (Hermesetas) in a solution of water supplemented with lemon juice concentrate (1:20 dilution). The volume of carbohydrate beverage was calculated to provide 1.5 g/kg body mass/h carbohydrate during the recovery period (every 15 minutes following the end of the sprint cycling exercise over a 150-minutes period), which approaches the maximal carbohydrate absorption rate of the gastrointestinal system.\textsuperscript{25} An identical volume of beverage was consumed in the placebo trial. A slightly lower carbohydrate intake (ie, 1.2 g/kg body weight/h, glucose/fructose ratio of 2:1) during the first hours following an exhaustive glycogen-depleting exercise bout was previously shown to highly stimulate muscle glycogen resynthesis.\textsuperscript{29} Participants were told to take a similar meal on the evening before each exercise session. They were asked to refrain from any food and caffeine intakes on the morning before the exercise sessions.

2.6 Neuromuscular testing of knee extensors

Neuromuscular testing consisting of electrically evoked torque and maximal voluntary contraction (MVC) was performed as described previously.\textsuperscript{30,31} The isometric torque of the knee extensors muscle was measured using an isokinetic dynamometer (System 3; Biodex Medical Systems) calibrated according to the manufacturer’s recommendations, with a correction for gravity performed using the
Biodex Advantage program (Version 4.X). The subjects sat upright in the dynamometer chair with the knee joint position at an angle of 60° (0° = full knee extension). Shank, trunk, and shoulders were stabilized by belts. Direct muscle stimulation was applied using two carbonized rubber surface electrodes (MARP Electronic), covered with a thin layer of electrode gel (ECG Gel; Ceracarta). One electrode (6 × 11 cm) was positioned transversely across the width of the proximal portion of the quadriceps femoris, and the second one (6 × 20 cm) covered the distal portion of the muscle above the patella. Caution was made to similarly position the electrodes during the two randomized trials. A standard electrical stimulator (MG 440; Medicor) was used to deliver 0.5-ms square wave pulses. The intensity of electrical stimulation was selected individually during a separate occasion (ie, at least 3-4 days before the experiments) by applying single pulses, and the current was increased until no increment in single twitch force could be detected by an additional 10% in current strength. Current stimulation trains of 1 second duration were used to determine the torques at 20 (P20) and 100 (P100) Hz. Low-frequency force depression (LFFD) was assessed from the P20/P100 ratio.

Two isometric MVCs of the knee extensor muscles were separated by a 20-seconds rest period. The subjects were verbally encouraged to exert and maintain maximal effort for 4-3 seconds. A 250 ms train of electrical pulses at 100 Hz was superimposed on the voluntary contraction 3-4 seconds into the MVCs. Central activation ratio (CAR), a measure of voluntary activation level, was calculated as the ratio of MVC into the MVCs. Central activation ratio (CAR), a measure of voluntary activation level, was calculated as the ratio of MVC into the MVCs. The highest value of MVC from the two attempts was used for further analyses.

2.7 Muscle biopsies and glycogen measurements

Needle biopsies from the non-dominant vastus lateralis muscle were taken by a trained medical doctor, as previously described.32,33 Briefly, after skin sterilization and local anesthesia with a subcutaneous injection of ~1.5 mL of 2% lidocaine (Grindeks), a 2-3 mm long cut with a scalpel tip was made in the skin and fascia. Biopsies were collected using an automatic biopsy device (DeltaCut; Pajunk Medizintechnologie GmbH). A 14-gauge disposable needle (DeltaCut; Pajunk Medizintechnologie GmbH) was inserted perpendicular to the muscle fibers, and three biopsy samples (~10 mg each) were collected at each time point (pre-exercise, post-exercise, and 180 minutes following the last cycling sprint). Muscle samples were immediately frozen in liquid nitrogen and stored at −80°C until analysis.

To prepare the muscle samples for analysis, 5-10 mg of frozen muscles was dissolved in 25 volumes of 2 mol/L NaOH for 50 minutes at 95°C. The muscle samples were vortexed every 15 minutes during this period. Homogenates were neutralized with an equal volume of 2 mol/L HCl. Protein concentration of the muscle homogenates (1:50 dilution) was determined by the Bradford method, following the manufacturer’s instructions (Bio-Rad). The muscle homogenates were then diluted eight times with distilled water. These diluted muscle homogenates (1:400) were loaded in duplicates (5 µL) to a 96-well plate. Muscle glycogen concentration was established using a fluorometric kit (ab65620; Abcam) in accordance with the manufacturer’s instructions. Adjustment of muscle glycogen for protein was performed by dividing the individual glycogen concentration by the individual protein concentration and then by multiplying by the mean protein concentration from all biological samples, as previously described.34 Muscle glycogen concentration was expressed as mmol glucosyl units/kg wet weight. The rate of muscle glycogen resynthesis (mmol glucosyl units/kg wet weight/h) was calculated by dividing the difference between the 180 minutes post-exercise values and the post-exercise values by three (ie, time in hours between the two time points).

2.8 Statistical analysis

Data are presented as mean ± standard deviation (SD). All statistical analyses were performed with Graphpad Prism Software (Graphpad Prism 8.1). Shapiro-Wilk tests were used to check normality before selecting the appropriate parametric or non-parametric statistical tests. Paired t tests were used to compare RPEmean during continuous exercise, HRpeak during sprint exercise and the rate of muscle glycogen resynthesis between the two experimental trials. Wilcoxon’s matched-pairs signed-rank tests were used to compare HRmean during continuous exercise and mean power during sprint exercise between the two experimental trials. For the analysis of the 20, 100 Hz torques, and MVC, two-way repeated measures analysis of variance (two-way RM ANOVA) were used to assess the effect of time and supplementation (CARB vs PLA). For the analysis of P20/P100, CAR, and muscle glycogen concentration, Friedman’s tests were used to assess the effect of time in both experimental trials, and Wilcoxon’s matched-pairs signed-rank tests with Bonferroni corrections were used to compare the CARB and PLA conditions at each time point. For the analysis of blood lactate and glucose concentration, a mixed-effects analysis was used due to missing data (1 missing value for blood lactate concentration and 2 missing values for blood glucose concentration). When appropriate, Sidak’s multiple comparison tests were performed to compare the CARB and PLA conditions at each time point. The α-level of significance was set at P < .05.
One subject was excluded from the analysis of muscle glycogen (Figure 2A,B) due to outlier values (ie, >2 SD) above the mean in the condition post-exercise for the CARB trial, that is, muscle glycogen concentration at post-exercise for the CARB trial was 55% of the pre-exercise value for this excluded subject, while it was on average ~5% of the pre-exercise value for the other participants.

3 | RESULTS

3.1 | Glycogen-depleting followed by sprint exercise session

The intensity of the continuous cycling exercise was in each subject set to 60% of VO2max in both the CARB and PLA experimental trials, hence giving similar mean power output and RPE (Table 1), although mean HR during the continuous exercise was slightly higher for the CARB trial compared with the PLA trial (+3%, \( P < .05 \)). During the six 30-seconds all-out cycling sprint bouts, the mean power output and peak HR were not significantly different between the PLA and CARB trials (Table 1). Note that the mean power output during the sprints was about 60% higher than the final power output in the VO2max test (4.26 ± 0.55 W/kg body mass).

Muscle glycogen concentration was not significantly different between the two randomized trials before exercise (Figure 2A). Muscle glycogen concentration was drastically decreased after the glycogen-depleting sessions to reach 6.8 and 2.7 mmol glucosyl units/kg wet weight during the PLA and CARB trials, respectively (Figure 2A). Three hours post-exercise, muscle glycogen concentration was significantly higher in the CARB trial than the PLA trial (44.0 and 22.8 mmol glucosyl units/kg wet weight, respectively; Figure 2A, \( P < .05 \)). The rate of muscle glycogen resynthesis during the 3-hours recovery period was significantly higher in the CARB trial than the PLA trial (13.7 and 5.4 mmol glucosyl units/kg wet weight/h, respectively; Figure 2B, \( P < .05 \)), demonstrating that CARB supplementation promotes muscle glycogen resynthesis after a glycogen-depleting exercise session. Moreover, blood glucose concentration increased in response to CARB supplementation (main effect, \( P < .001 \)) and was significantly higher than in the PLA condition from 30 minutes post-exercise (Figure 2C). The blood lactate concentration was markedly increased after the completion of the six all-out cycling sprints, and it progressively decreased during the recovery period (Figure 2D). A significant main effect of supplementation (\( P < .05 \)) was observed for the blood lactate concentration, but there was no difference between the PLA and CARB conditions at any time points.
3.2 Recovery of post-exercise neuromuscular function was not affected by CARB supplementation

Neuromuscular function was assessed before and during the 180-minutes post-exercise recovery period. The MVC torque was similarly decreased 15 minutes post-exercise (~30% decrease) in the two trials and did not fully recover over the post-exercise recovery period (effect of time, \( P < .001 \); Figure 3A). CARB supplementation had no beneficial effect on the recovery of MVC torque. Likewise, CAR was slightly reduced 15 minutes post-exercise (~15% decrease) in both conditions (effect of time, \( P < .01 \); Figure 3B), indicating slight CARB-independent reduction in central activation.

Representative 20 Hz torques are illustrated in Figure 4A, and mean data of 20 and 100 Hz torques are presented in Figure 4B,C. In both trials, 20 Hz torques were markedly decreased 15 minutes after the last sprint bout (~60% decrease) and were not completely recovered after 180 minutes (effect of time, \( P < .001 \); Figure 4B). Changes in 100 Hz torques were limited in both trials (Figure 4C), with only a slight decrease (5%-10%) observed 15 minutes after the exercise session. CARB supplementation affected neither 20 nor 100 Hz torques, and hence, there was no difference in the extent of PLFFD, as assessed by the P20/P100 ratio, between the two conditions (Figure 4D).

4 DISCUSSION

Prolonged low-frequency force depression (PLFFD) is a persisting fatigue-induced reduction in submaximal force observed in the post-exercise recovery period. Fatigue may cause PLFFD due to energy substrate depletion, as it is well known that muscle glycogen depletion is a major cause of fatigue-induced reductions in SR Ca\(^{2+}\) release and force loss during moderate-intensity endurance-type exercise.\(^7,15,17-18,35-37\) PLFFD may also occur after short-lasting high-intensity exercise where the decrease in muscle glycogen concentration is limited\(^20\) and the force depression would be due to glycogen-independent mechanisms, for instance, increased RONS production.\(^3,38\) Thus, the purpose of this study was to determine whether CARB ingestion, and consequently enhanced muscle glycogen resynthesis, would accelerate recovery of PLFFD induced by a combination of moderate-intensity glycogen-depleting and high-intensity sprint exercise. Using a randomized crossover study design, our results show that despite faster glycogen resynthesis, carbohydrate ingestion had no effect on the reversal of PLFFD when compared with placebo during the acute 3-hours post-exercise recovery period.

Glycogen depletion acts as the strongest stimulus for resynthesis, with the fastest rate of glycogen resynthesis occurring within the 0- to 6-hours post-exercise recovery period.\(^24-26\) Our fatiguing exercise protocol was similar to previously used protocols whereby prolonged moderate-intensity cycling exercise followed by high-intensity intervals elicited marked muscle glycogen depletion.\(^24,27\) Indeed, the current muscle glycogen depletion was severe, reaching 2%-4% of the pre-exercise value, which is similar to that previously reported with similar exercise protocols.\(^24,27\) In addition, other studies have shown that a moderate-intensity cycling exercise of 60 minutes induced 60%-70% muscle glycogen depletion\(^39\) while four 30 seconds all-out

### TABLE 1 Power, HR, and RPE from continuous cycling exercise and all-out sprint exercises

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<thead>
<tr>
<th></th>
<th>Placebo Trial</th>
<th>Carbohydrate Trial</th>
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<tr>
<td><strong>Continuous exercise</strong></td>
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<tr>
<td>( \text{Power}_{\text{mean}} ) (W/kg bm)</td>
<td>2.25 ± 0.26</td>
<td>2.21 ± 0.30</td>
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<tr>
<td>( \text{HR}_{\text{mean}} ) (bpm)</td>
<td>150 ± 12</td>
<td>145 ± 11*</td>
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<tr>
<td>( \text{RPE}_{\text{mean}} )</td>
<td>14.8 ± 1.0</td>
<td>15.1 ± 0.6</td>
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<tr>
<td><strong>Sprint exercise</strong></td>
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<tr>
<td>( \text{Power}_{\text{mean}} ) (W/kg bm)</td>
<td>6.85 ± 0.48</td>
<td>6.92 ± 0.63</td>
</tr>
<tr>
<td>( \text{HR}_{\text{peak}} ) (bpm)</td>
<td>167 ± 10</td>
<td>169 ± 9</td>
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*Note: Data are shown as means ± SD. HR\(_{\text{peak}}\) was calculated from the average of the peak HR of each cycling sprint. N = 9.

Abbreviations: bm, body mass; HR, heart rate; RPE, rated perceived exertion. *\( P < .05 \), significantly different compared with the placebo condition. It should be noted that mean power output (Power\(_{\text{mean}}\)) was set before the continuous cycling exercise and was therefore identical for both trials.

**FIGURE 3** Maximal voluntary contraction (MVC) torque (A) and central activation ratio (CAR) (B) before exercise and during the post-exercise recovery period supplemented with carbohydrate (CARB) or placebo (PLA). Data are shown as means ± SD. Pre-ex, Pre-exercise. N = 9.

\( **P < .01 \), ***\( P < .001 \), significant main effect of time
cycling exercise induced ~20% muscle glycogen depletion.\textsuperscript{20} Thus, combining 60 minutes moderate-intensity cycling exercise with six 30 seconds all-out cycling exercise is likely to result in a muscle glycogen depletion of the magnitude observed in the present study, especially since different fiber types would be activated in the two exercise modalities (predominantly slow-twitch fibers with moderate-intensity exercise, and both slow- and fast-twitch fibers with all-out sprints).

Despite the significant increase in glycogen resynthesis, the ingestion of carbohydrates did not improve the recovery of MVC torque or involuntary muscle contractile performance (20 Hz, 100 Hz, P20/P100) compared with the placebo. The decrease in MVC force was accompanied by a slight decrease in CAR, indicating some decline in the ability to voluntarily activate the exercised muscles. This deficit in central activation was not affected by carbohydrate supplementation, and it was too small to have any notable impact on the observed PLFFD.

Prolonged low-frequency force depression was readily apparent with a ~55% decrease in the 20/100 Hz ratio post-exercise. Intriguingly, the enhanced glycogen resynthesis associated with carbohydrate ingestion did not result in a faster reversal of PLFFD than with placebo. Thus, the current results do not support a role of carbohydrate ingestion in the reversal of PLFFD in the acute 3-hours post-exercise recovery period, which differs from several previous studies showing improved recovery with carbohydrate supplementation after glycogen-depleting exercise.\textsuperscript{7,36} For instance, the results from a previous study in mouse intact muscle fibers show that after low-intensity glycogen-depleting stimulation, low frequency (30 Hz force) recovers in the presence of 5 mmol/L glucose but not in the absence of glucose.\textsuperscript{7} However, full glycogen repletion occurred after only 1 hour of recovery in mouse muscle, whereas in the current study, glycogen was only recovered to 45% vs 20% of the pre-exercise value in the CARB vs PLA trial after 3 hours of recovery. Thus, the rate of glycogen resynthesis is markedly lower in human than in mouse muscle and it is possible that a longer post-exercise recovery period with CARB supplementation, and hence more complete glycogen recovery, could reveal an effect on the reversal of PLFFD. Furthermore, an important difference between our present study and these previous studies is that our stimulation protocol also included six 30-seconds maximum sprint cycling bouts.

Reactive oxygen and nitrogen species can cause long-lasting oxidative modifications to muscle proteins and increased RONS production has previously been established as a major cause of PLFFD following fatiguing exercise.\textsuperscript{3,5-6,8-10,18} The contraction-mediated generation of RONS increases with increasing contraction intensity,\textsuperscript{23} with the greatest RONS produced during high-intensity exercise.\textsuperscript{3} High-intensity interval exercise in itself does not cause a major decrease in muscle glycogen content,\textsuperscript{20,40} and yet it was previously shown that PLFFD was induced by high-intensity interval cycling exercise and it persisted for up to 24 hours.\textsuperscript{3} In the current study, high-intensity interval cycling was performed after 1 hour of moderate-intensity cycling. Thus, it is likely that increased RONS production during the cycling sprints induced PLFFD via glycogen-independent mechanisms, which would explain why enhanced glycogen resynthesis did not improve force recovery. However, in this study, we did not perform any
direct RONS-related measurements and can therefore not exclude that the cycling sprints induced PLFFD via mechanisms other than increased RONS.

5 | CONCLUSION

We here provide in vivo results demonstrating that post-exercise carbohydrate ingestion does not accelerate the acute recovery of PLFFD induced by a combination of moderate-intensity glycogen-depleting and sprint cycling exercise. Thus, with this type of exercise, we believe that PLFFD is mainly caused by RONS-dependent mechanisms that are not mitigated by enhanced restitution of muscle glycogen stores.

6 | PERSPECTIVES

Our results clearly indicate the necessity to consider the complexity of integrative, physiologically relevant conditions when investigating PLFFD. For instance, the design of our exercise protocol mimics that experienced during many endurance-type sports where prolonged moderate-intensity exercise ends with a final high-intensity spurt. Our findings indicate that post-exercise carbohydrate supplementation does not improve the initial recovery phase of submaximal force in such endurance-type sports, where the final spurt is likely to promote PLFFD via glycogen-independent mechanisms. However, this does not exclude the possibility that there are other benefits of enhancing muscle glycogen resynthesis in such situations; for instance, carbohydrate supplementation may increase the endurance capacity for repeated bout exercise performance. Moreover, carbohydrate supplementation has been shown to improve force recovery when PLFFD is induced by prolonged low-intensity exercise that does not involve any sprint activities and hence does not trigger additional mechanisms by which PLFFD can be induced. Thus, future studies aimed at finding strategies to improve force recovery after PLFFD-inducing exercises should consider the fact that mechanisms underlying PLFFD are multifaceted.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

AJC, SK, MB, HW, and TV were involved in the study design. SK, MB, AS, TC, and TV were involved in data collection, and data analysis was performed by AJC, TC, SK, MB, HW, and TV. AJC and TC drafted the manuscript. AJC, TC, SK, MB, HW, and TV edited and revised manuscript. SK, MB, AS, and TV assisted with recruitment of the subjects and data collection. All authors approved the final version of the manuscript.

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